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Environment and Gut Morphology influence Microplastic Retention in Langoustine, *Nephrops norvegicus*

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ABSTRACT

Over the past twenty years microplastic pollution has been recorded in all major marine habitats, and is now considered to be of high environmental concern. Correspondingly, the number of reports of microplastic ingestion by marine species is increasing. Despite this, there are still relatively few studies which address the uptake and retention of microplastic in wild populations. Langoustine, *Nephrops norvegicus*, sampled from the Clyde Sea Area, have previously been seen to contain large aggregations of microplastic fibres. The large proportion of contaminated individuals and size of the microplastic aggregations observed suggests that *Nephrops* are at high risk of microplastic ingestion. In this study the levels of ingested microplastic in populations of *N. norvegicus* from the Clyde Sea Area, North Minch and North Sea are examined. Animals in the near-shore, Clyde Sea population showed both a higher percentage of microplastic containing individuals and much greater weights of microplastic retained in the gut. *N. norvegicus* revealed that only a small percentage of individuals from the North Sea and Minch contained microplastic, predominantly single strands. An expanded sample from the Clyde Sea Area was examined to identify the factors influencing microplastic retention. This revealed that males, larger individuals, and animals that had recently moulted contained lower levels of microplastic. The presence of identified food items in the gut was not seen to correlate with microplastic loads. Observations of microplastic in the shed stomach lining of recently moulted individuals and the lack of aggregations in wild-caught individuals suggests that ecdysis is the primary route of microplastic loss by *N. norvegicus*. Therefore the large aggregations observed in wild-caught animals are believed to build up over extended periods as a result of the complex gut structure of *N. norvegicus*.

Keywords: microplastic; pollution; monitoring; Decapoda;

Capsule: Analysis of microplastic aggregation by wild *Nephrops norvegicus* from three populations determined that location and moult stage have the largest effect on aggregation.

1. Introduction

The current scientific focus on the distribution and fate of microplastic pollution has led to numerous studies of its effects on marine communities. Due to the resistance of polymers to degradation and their relative buoyancy, plastics are able to persist for long periods in the marine environment, and be carried far from their source (Barnes et al., 2009). As a result, microplastics have been reported in environments far from anthropogenic activities. (Barnes et al., 2009; Van Cauwenberghe et al., 2013).

Many of these observations of marine microplastic have shown a high degree of heterogeneity in microplastic distribution (Ryan et al., 2009). The greatest densities of microplastic debris have been reported from the centres of gyres (Moore et al., 2001), lagoons (Vianello et al., 2013), and in coastal sediments (Claessens et al., 2011). These apparent at risk areas are the result of a number of environmental factors, such as wind direction, currents, and bathymetry (Dixon and Dixon, 1983; Moreira et al., 2016; Shaw and Mapes, 1979). Proximity to sources of microplastic pollution have also been seen to have a significant impact on local abundance (Reddy et al., 2006).

Despite the recent increase in available literature, much of the evidence on plastic uptake by animals relates to the ingestion of macroplastic by large marine vertebrates, such as birds (van Franeker et al., 2004) and turtles (Lutz, 1990). Sampling of fish collected in trawls has also shown that a range of fishes also take up both macro- and microplastics from the environment (Boerger et al., 2010; Lusher et al., 2013). Unfortunately due to the large ranges over which these species forage, and the uncertainty over the length of time plastic is retained in the gut, it is difficult to draw a conclusion as to the relationship between environmental and ingested plastics.

Many of the observations of plastic ingestion by invertebrates stem from laboratory investigations. In this way, uptake of microplastics has been observed in blue mussel, *Mytillus edulis* (Browne et al., 2008; Farrell and Nelson, 2013; von Moos et al., 2012), shore crab, *Carcinus maenas* (Farrell and Nelson, 2013), sandhoppers, *Talitrus saltator* (Ugolini et al., 2013), lugworm, *Arenicola marina* (Browne et al., 2013), and echinoderms (Graham and Thompson, 2009). Shore crabs have also been seen to take in plastic microspheres through the gills during normal respiration (Watts et al., 2014), and trophic links have been indicated by the transfer of microbeads from mussels (Farrell and Nelson, 2013).

Fewer studies examine the uptake of microplastic by wild-caught invertebrates; however, the level of uptake observed appears to support the findings of laboratory investigations. Large numbers of contaminated individuals have been recorded amongst crustaceans; for example 63% of brown shrimp, *Crangon cragon*, sampled from the English Channel and southern North Sea were seen to contain microplastics (Devriese et al., 2015), as were 82% of langoustine, *Nephrops norvegicus*, from the Clyde Sea Area (Murray and Cowie, 2011). Lower levels were observed amongst gooseneck barnacles, *Lepas* spp., with 33.5% of 385 individuals sampled from the North Pacific Sub-tropical Gyre seen to contain microplastic (Goldstein and Goodwin, 2013).

N. norvegicus is a species of great importance to the UK fishing industry. In 2014 it accounted for a fifth of the weight of shellfish landings by the UK fleet and a third of the value, at 30 thousand tonnes and £99 million. This substantial sum made it the second most important fishery in the UK in 2014. Murray and Cowie's examination of 120 *N. norvegicus* from the Clyde Sea indicated that large aggregations of microplastic are found in a significant proportion of the population. Unlike the vertebrates previously seen to take up plastics in the wild, *N. norvegicus* feed within a small area around their burrows; thus the level of contamination in wild caught animals is potentially indicative of the amount of microplastic in the surrounding environment.

The Clyde Sea Area is an enclosed waterbody in close proximity to numerous potential microplastic sources, a combination of factors which suggests a high abundance of environmental microplastics. However, geographically separated populations of *N. norvegicus* are exposed to very different bathymetric conditions and anthropogenic influences. The variation in distance from sources of litter suggests that the average intake of microplastic by *N. norvegicus* populations in other locations may be much lower.

This study examines the occurrence of microplastic in *N. norvegicus* in Scottish waters. The work aims to determine whether the high levels of microplastic observed by Murray and Cowie (2011) are representative of those in other populations. Analysis of the environmental and biological factors related to microplastic levels in the three studied populations is used to identify the factors responsible for the aggregation of ingested microplastic.

2. Materials and Methods

2.1. Microplastic in Scottish Nephrops norvegicus

N. norvegicus were collected from three sites in North and West Scotland; the Clyde Sea Area (CSA), the North Minch (NM), and the North Sea (NS) (Fig. 1). In the CSA four trawls were taken at Skelmorlie Bank and in the Main Channel at depths between 58 and 110 metres in May, June and August (Fig. 2). Sampling was carried out using otter trawls rigged with 50mm mesh. To reduce the potential for uptake of fibres from the sampling net was reduced by only carrying out short trawls. Individuals were frozen immediately on landing to prevent digestion of the gut content.

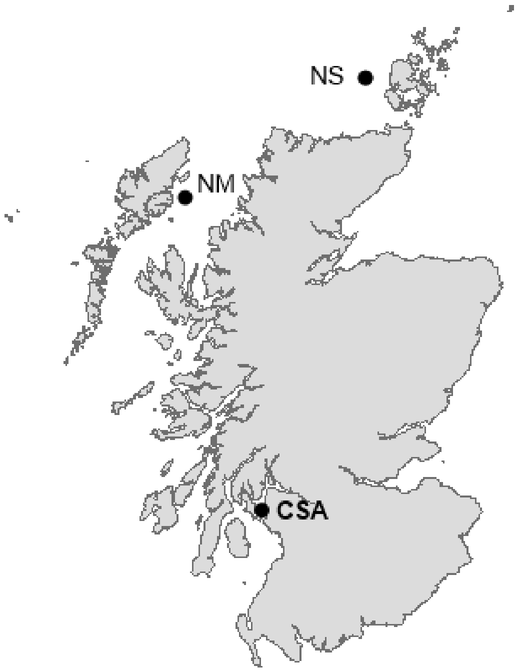

Fig. 1. Trawl Locations in the North Sea (NS) -3º49.07'E, 59º03.39'N, North Minch (NM) -6˚09.13'E, 58˚08.57'N, and Clyde Sea Area (CSA) -4.9751E, 55.7892N

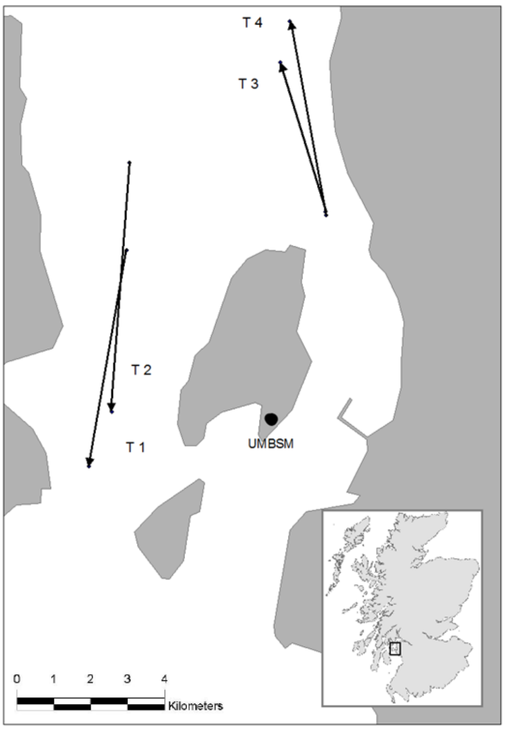

Fig. 2. Showing trawl locations in the Clyde Sea Area in relation to the University Marine Biological Station Millport (UMBSM). T1: 16/06/2011 -4.8903E, 55.7998N ~ -4.9093E, 55.8463N, T2: 16/06/2011 -4.9751E, 55.7892N ~ -4.9872E, 55.7368N, T3: 08/07/2011 -4.8905E, 55.8005N ~ -4.9127E, 55.8362N, T4: 11/08/2011 -4.9755E, 55.8105N ~ -4.9131E, 55.8472N

Animals were defrosted prior to dissection, and their sex, moult stage, and carapace length recorded. Moult stage was determined by testing the hardness of the thorax, directly behind the eyes. Intermoult individuals could be identified by their hard carapaces, while recently moulted individuals have a jelly-like carapace, and those of animals immediately prior to and post moult is papery (Farmer, 1973). The carapace of the individual was then removed and the muscle of the thorax and tail separated to allow the removal of the stomach and gastric tract, which was preserved in 80% ethanol (Murray and Cowie, 2011).

The content of each gut was examined under a stereo microscope to determine the volume and identity of natural prey and presence of potential microplastics. Gut contents were examined in subsets of approximately 0.5 ml (a level spatula) to ensure that the detectability of plastic in the gut contents was not impacted by the volume of food. Plastic materials were categorised as either pre-production pellets, fragments, films or fibres. Aggregations of fibres were grouped into the following subcategories; up to five strands, strands and a loose ball of fibres, and a tight ball of multiple fibres (Murray and Cowie, 2011). A Mettler MX5 balance (Mettler-Toledo international Inc., Columbus, USA) was then used to record the weight of plastic recovered from each individual to five decimal places. Prior to weighing, any algae tangled among the plastic fibres were removed and the samples air dried for 48 hours. Each sample was weighed three times and a mean taken.

2.2 Identification of Microplastic

FT-IR spectrometry was used to identify a sub-set of 100 suspected plastic items. Tangled fibres were separated for individual analysis and all samples were rinsed in distilled water and allowed to air dry to ensure the cleanest possible spectrum. Samples were analysed using a Shimadzu 8400s spectrometer and the resulting spectra were compared to those of a range of known polymer standards to confirm their identity. The percentage of successfully identified plastics was used to calculate the actual number of microplastic items recovered.

2.3. Duration of Microplastic Retention

Previous examination of 120 individuals from the CSA indicated lower plastic contamination in recently moulted individuals (Murray and Cowie, 2011). To establish whether microplastics are lost during ecdysis a two month feeding trial was carried out. Ten recently moulted female *N. norvegicus* were placed into individual tanks and fed with a daily ration of 0.5 g of squid mantle, seeded with five 0.5mm strands of polypropylene (PP) rope. After the first month, bilateral eye ablation was used to induce moult (Fingerman, 1987). Feeding with microplastic seeded squid was continued until the individual had achieved ecdysis. The shed gut lining was recovered and the moulted individual frozen for gut content analysis as described above.

2.4. Statistical Analysis

Analysis of the factors affecting microplastic accumulation by *N. norvegicus* was carried out using Minitab 15. The relative frequencies of microplastic containing animals in the three populations were examined using Chi-squared analysis.

The data from the CSA sample was used to determine both the factors affecting microplastic ingestion, and those affecting the weight of retained microplastic. The statistical software R, version 3.0.2, was used to relate the microplastic data to carapace length, sex, moult stage, trawl number, sampling site, and the presence and type of food. Factors associated with the likelihood of plastic occurrence in *N. norvegicus* were determined by fitting a binary logistic model (BLM). The factors responsible for the weight of retained plastic were examined using a generalised linear model (GLM).

3. Results

Trawl samples returned 1450 animals for dissection and analysis. Those from the North Minch (150 animals) and North Sea (300 animals) were all male, whilst the larger sample of individuals from the CSA (1000 animals) was separated into 50% males and females. Of the total 1450 individuals, 975 (67%) were seen to contain microplastic, predominantly microfibres.

The fibres recovered were a range of colours and thicknesses, the exact proportions of which could not be determined due to the highly tangled aggregations. Of the samples yielding sufficiently clear spectra for analysis 94% were confirmed as plastics. Nylon and polypropylene were the most frequently observed polymers, and made up 37.2%, 29.8% and 12.8% of the analysed plastic, respectively. Smaller amounts of polyethylene - mainly from ingested films - and PVC were also recovered.

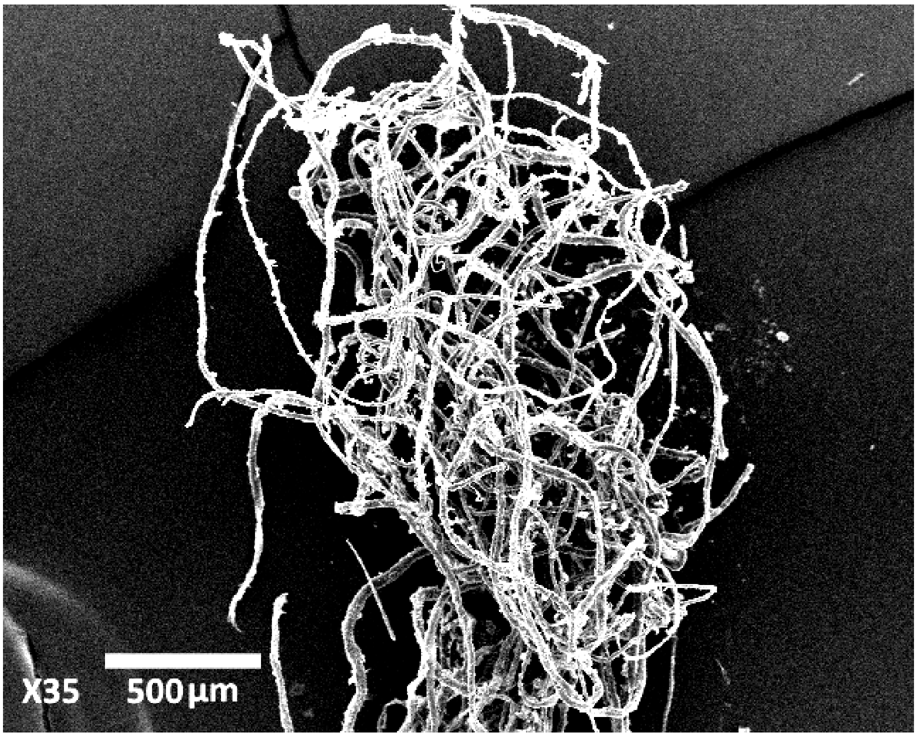

Fig. 3. Aggregation of plastic fibres recovered from the foregut of a female *Nephrops norvegicus* from the Clyde Sea Area

3.1. Local Variation in Microplastic Uptake

Variation was seen in the proportion of individuals at each site which contained microplastics (Table 1). Chi-squared analysis of the number of contaminated individuals at each site indicated a significant difference between the three locations ($P < 0.001$, $X^2 = 572.756$, df =10). This disparity is driven by individuals sampled from the Clyde.

Table 1

The occurrence and retention of microplastics by Scottish *Nephrops norvegicus* stocks

Site	Sample Size	Proportion of Sample seen to Contain Microplastics	Maximum Microplastic Weight (mg)	Average Microplastic Weight (mg)
Clyde Sea Area	1000	84.10%	0.09	-
North Minch	150	43.00%	0.01	>0.01
North Sea	300	28.70%	0.80	0.40

The most commonly isolated plastics were fragmented fibres. Other plastics found were mainly films, although one pre-production nib was isolated. In the offshore populations, contaminated individuals contained a maximum of 5 fibres, whilst 41% of the CSA were seen to have aggregated tangled "Balls" of multiple fibres and algae (Fig. 3).

3.2. Factors Affecting Microplastic in the Gut of N. norvegicus from the Clyde Sea

Trawl depths in the CSA ranged from 74 – 115m in the Main Channel and 60 – 75m in the Fairlie Channel; sediment analysis at the two sites revealed average grain sizes of 0.18mm and 0.166mm respectively. The carapace length of individuals in the CSA sample ranged from 19.8 to 59.1mm, and was found to be normally distributed when examined using Kolmogrov-Smirnov analysis ($P < 0.010$). The proportion of individuals at each moult phase differed between males and females, possibly the result of reduced moult frequency in mature females (Farmer, 1973). The examination of identifiable prey items indicated a diet dominated by bivalve molluscs and crustaceans, with *N. norvegicus* carapace regularly observed. These two categories made up 74.1% of the identifiable gut contents, with the rest being comprised of fish bones, echinoderms and polychaetes. Variation in the aggregation and weight of plastic was observed in individuals recovered from the four trawls, with lower weights of plastic seen later in the year (Table 2).

Table 2

The occurrence and retention of microplastics by *Nephrops norvegicus* in the Clyde Sea Area

Trawl	Site	Date	Total Animals	Upto five fibres	Between 5 and a loose ball of fibres	Ball of fibres	Individuals seen to contain films	Average of Plastic Weight (mg)
1	Main Channel	16/06/2011	383	19.32%	21.40%	51.69%	3.39%	0.66
2	Fairlie Channel	16/06/2011	184	32.06%	22.28%	36.95%	3.80%	0.47
3	Main Channel	08/07/2011	275	40.36%	19.27%	18.18%	3.27%	0.20
4	Fairlie Channel	11/08/2011	158	33.54%	6.96%	19.62%	12.65%	0.28

The results of the BLM identified moult stage, date of trawl, and carapace length as having a significant impact on the likelihood of plastic contamination in *N. norvegicus*. Recently moulted ("jelly" carapace) individuals were seen to be less likely to contain plastics than those at intermoult ("hard" carapace) ($z= -6.112$, $P<0.001$). Carapace length was also seen to effect the likelihood of plastic presence, with smaller individuals more likely to contain microplastics ($z= -1.829$, $P<0.05$); however, the observed relationship was of lower significance than that of moult stage and trawl date.

While there was a significant difference in the occurrence and aggregation of plastic recovered from *N. norvegicus* from different geographical areas, there was no difference observed between the trawl locations within the CSA. The only non-biotic factor observed to have a significant impact on whether plastic was present within the gut was the season in which the animals were collected; with lower likelihood of plastic contamination in tows carried out in June; trawl three ($z= -3.675$, $P<0.001$), and August; trawl 4 ($z= 4.3$, $P<0.001$). This variation is believed to be due to a reduction in the number of recently moulted individuals later in the year.

The results of the GLM analysis of the factors associated with variation in the weight of microplastic returned a similar response to that of the BLM of plastic occurrence. The results indicated that the moult stage of the individual was significantly related to the weight of plastic present ($P<0.001$), this was driven by lower weights of plastic in recently moulted individuals (Fig. 4). Females were seen to retain greater weights of plastic than males ($t= 4.245$, $P<0.001$) (Fig.5). A significant negative relationship was also observed between the proportion of gut occupied by food and the weight of recovered plastic ($P<0.001$); individuals recorded as having no food in the foregut were observed to have the highest microplastic load. Sampling trawl had the highest influence over the amount of plastic retained ($P<0.001$), this was driven by a low average plastic weight recovered in animals from trawls three and four.

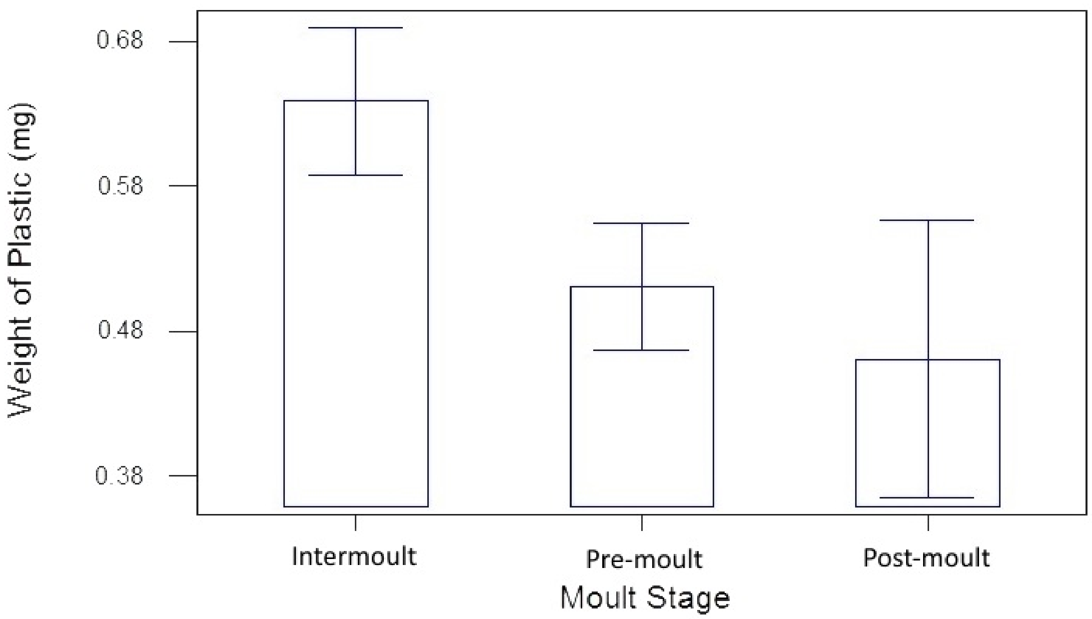

Fig. 4. The weight of plastic (mg) recorded in *Nephrops norvegicus* at each moult stage

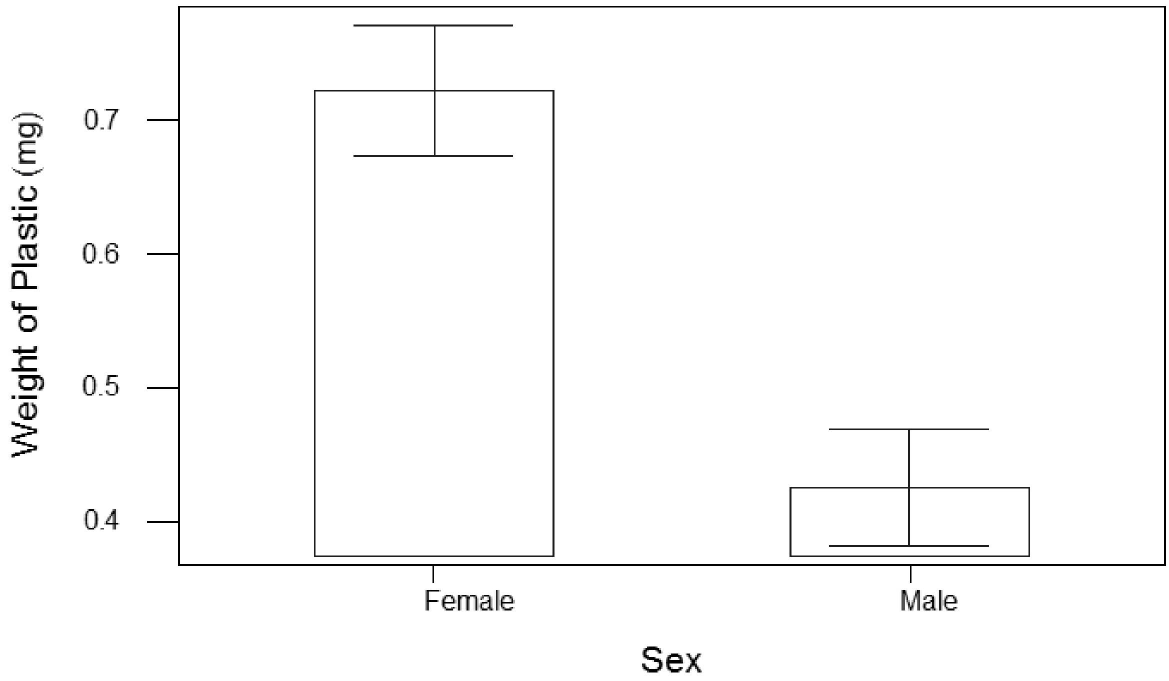

Fig. 5. The weight of plastic (mg) recorded in male and female *Nephrops norvegicus*

3.3. Plastic Lost at Moult

Seven of the 10 animals subjected to eye ablation moulted within the two month experimental period. During the feeding period the animals did not consume the whole food ration each day, and so the total number of fibres ingested could not be definitively stated; however, when the gut linings were examined under stereo microscope, five were seen to contain microplastics. Stomach content analysis carried out on all post moult individuals revealed no remaining plastics in the foregut, whereas plastic aggregations were observed in the three un-moulted individuals.

4. Discussion

Despite the number of studies into the distribution of microplastics, there are few that look at their ingestion in benthic habitats (Reddy et al., 2006; Van Cauwenberghe et al., 2013). Our results demonstrate microplastic uptake by *N. norvegicus* from each of the sampled locations. The most commonly observed plastics in all three populations were fibres, indicating either that these are the most abundant plastics at all sites or that fibres are more easily ingested. In previous studies of microplastic in the marine environment, fibres have been the dominant plastic category .

These fibres varied in colour, thickness and degree of wear, and are believed to originate from a range of sources. FT-IR analysis of single micro-fibres proved highly laborious, occasionally resulting in unclear, 'noisy' results – an issue also stated by Gallagher et al. (2015), however this did reveal 96% success rate in visual identification of plastics. This revealed a mix of polymers. Many of the observed fibres may have entered the CSA from the River Clyde and are potentially the released from clothes washing as outlined in Browne et al. (2011). Trawl nets may release plastics into the CSA both from regular use and the breakdown of lost gear; however, the number of blue and orange fibres observed was comparatively low.

4.1. Local Variation in Microplastic Uptake

The frequency of microplastic occurrence and level of aggregation observed in the CSA support that previously recorded by Murray and Cowie (2011) from a smaller sample size; however, it is apparent from the results that *N. norvegicus* from the North Minch and North Sea have substantially lower microplastic loads. The disparity in microplastic uptake is believed to be caused by the CSA's relative proximity to microplastic sources, resulting in locally raised concentrations of environmental microplastics. As such, areas close to high levels of human activity - such as estuaries and enclosed water bodies - may be thought of as high risk, and animals living there as having a greater likelihood of microplastic uptake. Within the Clyde sea there were significant differences in microplastic load between sample trawls; however, previous examination of the average abundance of microplastic in the sediments of the North Channel and Fairlie Channel – 45.5 and 42.2 plastic items per kilogram respectively – showed no significant variation (Welden, unpublished data). It is believed that the variation observed can be attributed to biotic differences.

The current route by which microplastics enter the food chain is unclear; however, many species of invertebrates (Devriese et al., 2015; Ugolini et al., 2013; Van Cauwenberghe and Janssen, 2014) and fishes (Boerger et al., 2010; Lusher et al., 2013; Lusher et al., 2015) have shown some degree of contamination. In the marine environment, interactions between animals and microplastic may occur in a number of ways; when examining plastic contaminated fish Lusher et al (2013) suggest that plastic is taken up accidentally during feeding, whereas Boerger et al (2010) indicate that microplastics may be actively consumed due to their resemblance to planktonic prey. Planktonic crustaceans have also been seen to actively ingest plastics, although some species appeared able to discriminate against larger polystyrene beads (Bern, 1990).

N. norvegicus act as both scavengers and carnivores, and may take up plastic during feeding or burrowing activities. Other crustaceans, such as crabs, have comparable feeding methods, and may be at a similar risk of microplastic loading. The shore crab *C. maenas* has been seen to take in microplastic spheres from contaminated mussel, *M. edulis* (Farrell and Nelson, 2013; Watts et al., 2014), and through the gills during respiration (Watts et al., 2014), as well as fibres from prepared mussel/gelatine blocks (Watts et al., 2015).

In a study of the impact of feeding mode, filterers such as bivalves were found to ingest the highest levels of microspheres (Setälä et al., 2015); if this is also true of fibres, molluscs in the CSA would experience a higher frequency of microplastic ingestion than that observed here. However, this relies on microplastic being obtained directly from the environment and does not take into account potential bioaccumulation. *N.* norvegicus are opportunistic scavengers, consuming a range of prey species. Bioaccumulation would rely on animals consuming multiple small prey animals whole, consuming their plastic load in the process. The observations of gut contents reported here revealed a high percentage of larger animals, such as mollusc and crustaceans, which were partially consumed. The potential for bioaccumulation is currently obscured by numerous sources of uncertainty; further research into the trophic transfer of microplastic and its retention by a range of species is required before the true risk can be established.

4.2. Biological Factors Influencing Microplastic Retention

The volume of microplastic recovered from *N. norvegicus* in the CSA reveals that aggregations are held in the foregut for extended periods of time. The statistical analysis of the factors responsible for high microplastic weights indicated that sex, size, and moult stage have the greatest influence on aggregation.

The negative relationship between body size and microplastic loads may be the result of the gut morphology of *N. norvegicus*. The digestive tract of crustaceans is relatively complex that compared to other invertebrates. The gastric mill is a set of chitinous plates found in the foregut, at the entrance to the hindgut (Farmer, 1975). The shape of these plates and the narrowing at the entrance to the hindgut may prevent microplastic from being egested with natural food-stuff. Previous work examining the morphology of the gastric mill in relation to carapace length has shown that the gaps in the mill increase with growth (Welden et al., 2015). The increased size of the gaps between plates of the mill may allow a greater amount of microplastic to be lost by egestion in larger individuals.

This link between size and microplastic loss may also explain the relationship seen between sex and microplastic aggregation. Female *N. norvegicus* grow at a slower rate than males due to a decreased moult frequency. As a result they have smaller gastric mills which would prevent the egestion of microplastics that could be passed by larger, male conspecifics.

The presence of fibres in the discarded gut lining of moulted *N. norvegicus* indicates that microplastic can be lost at ecdysis. This supports the outputs of both the BLM of microplastic occurrence and the GLM of factors influencing the weight of microplastic retained in the gut. Consequently, the authors believe moulting to be the lead cause of microplastic loss in langoustine.

Of the biological factors linked to the retention of microplastic it is likely that the size of the gastric mill has the greatest influence on the retention of microplastics; particularly in larger size classes. The significantly lower weight of microplastic in recently moulted individuals from the Clyde indicate that *N. norvegicus* rid themselves of large plastic aggregations at moult, this was supported by the observed plastic in the shed guts of animals subjected to eye ablation. These two factors are believed to have the greatest effect on the weight of retained microplastic. The discrepancy in microplastic observed between male and female langoustine is believed to be the result of increased moult frequency in male langoustine and, as male langoustine are generally larger, increased size in the gastric mill.

4.3. Potential Impacts of Microplastic Retention

The large aggregations of microplastic fibres observed in the CSA population indicate that *N. norvegicus* in this area are at increased risk of the biological impacts of plastic ingestion. Previous studies have shown a number of effects of ingested plastic on an animal's fitness. These include false satiation, previously described in seabirds (Ryan, 1988) and turtles (Lutz, 1990; McCauley and Bjorndal, 1999), and nutrient dilution - preventing the assimilation of ingested foods (McCauley and Bjorndal, 1999).

Although different in their mechanics, both of these conditions cause a reduced nutritional state and have been seen to result in starvation. For example, in the lugworm, *Arenicola marina*, plastic ingestion negatively affected feeding rate, leading to reduced body mass (Besseling et al., 2012). In addition to their impact on the body condition, plastics also carry hydrophobic contaminants (Teuten et al., 2007; Teuten et al., 2009). Regular ingestion and retention may result in pollutants transferring from plastic to the organism (Besseling et al., 2012).

The results presented above describe a negative relationship between microplastic weight and stomach fullness. This may indicate reduced feeding as a result of false satiation. Using the relative proportions of the identified polymers we calculated a mean specific gravity for the plastics in *N. norvegicus* in the CSA. From this it was possible to calculate an approximate mean volume of $0.68mm^3$ of aggregated plastic per contaminated individual. The calculated volume of the largest recorded aggregation was $9.40mm^3$. This volume may appear low on first examination; however the size of the observed aggregations were increased by trapped natural materials. An individual of 20mm carapace length if expected to have a gut volume of $0.806cm^3$ (Welden et al., 2015), and the combined plastic and algae aggregations observed took up to ten percent of the foregut. Whilst *N. norvegicus* are highly tolerant to starvation (Mente, 2010), long periods of retention may cause reduced (or even negative) growth.

Due to their smaller size and reduced moult rate at maturity, female *N. norvegicus* would retain plastic for up to twice as long as males, making them more likely to contain high plastic loads. Reduced body mass, is known to lower fecundity in a number of crustacean species (Beyers and Goosen, 1987; Hines, 1991; Lizárraga-Cubedo et al., 2003), including *N. norvegicus* (Abellô and Sardá, 1982). In European Lobster, *Homarus gammarus*, smaller individuals have also been shown to have smaller eggs (Tully et al., 2001). In this way, sub-lethal microplastic loads may have impacts at the population level.

N. norvegicus is a species of high economic importance in Europe. The impacts of microplastic ingestion on fitness and fecundity may impact the viability of nearshore fisheries. As a result of this uncertainty, the authors consider further examination of the impact of microplastic on the fitness of *N. norvegicus* to be of high importance.

5. Conclusion

It is clear from the results presented that *N. norvegicus* from nearshore habitats exhibit significantly higher microplastic abundance in them than those located in areas further from anthropogenic inputs. In addition to the much lower percentage of individuals seen to contain plastic, the large aggregations recorded in Clyde Sea animals were not observed in those from the North Sea and North Minch.

As well as the effect of location on plastic uptake, the individuals in the CSA sample indicate that size, sex and moult stage significantly influence microplastic loads. The ability of *N. norvegicus* to routinely expel microplastic aggregations along with the gut lining at moult reduces the negative effect of their complex gut morphology to some extent; however, the possibility of a 12 month microplastic exposure period suggests a high probability of associated negative impacts.

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